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ABSTRACTS

PART I

Abstracts 1–3006

536

INHIBITION OF Na^+/H^+ EXCHANGE PRESERVES VIABILITY, RESTORES MECHANICAL FUNCTION, AND PREVENTS THE pH PARADOX IN REPERFUSION INJURY TO CULTURED RAT NEONATAL MYOCYTES. (I.S. Harper, J.M. Bond, E. Chacon, J.M. Reece, B. Herman, and J.J. Lemasters). Dept. Cell Biol & Anat, Univ. of North Carolina, Chapel Hill, NC 27599, and Exp Biol Programme, Medical Research Council, Tygerberg 7505, South Africa.

Rat neonatal myocytes exposed to 2.5 mM NaCN and 20 mM 2-deoxyglucose at pH 6.2 (chemical hypoxia) quickly lose viability when pH is increased to 7.4, with or without washout of inhibitors ('pH paradox', (*BBRC* 179, 798). Here, we evaluated the effect of two Na^+/H^+ exchange inhibitors (dimethylamiloride and HOE694) and a Na^+/Ca^{2+} exchange inhibitor (dichlorobenzamil) on pH-dependent reperfusion injury. Intracellular Ca^{2+} and mitochondrial $\Delta\Psi$ were monitored by laser scanning confocal microscopy of myocytes co-loaded with Fluo-3 and tetramethylrhodamine methylester. After 30-60 min of chemical hypoxia at pH 6.2, mitochondria depolarized and Ca^{2+} began to increase. Ca^{2+} reached levels over 2 μM by 4 h. Washout of inhibitors at pH 7.4 (reperfusion) with or without dichlorobenzamil killed most cells within 60 min, despite a marked reduction of Ca^{2+} in dichlorobenzamil-treated cells. Reperfusion in the presence of 75 μM dimethylamiloride or 20 μM HOE694 prevented cell death. HOE694-treated cells recovered mitochondrial $\Delta\Psi$ before normal Ca^{2+} was restored. Hypercontracted myocytes re-extended over a 24 h period. By 48 h, most cells contracted spontaneously and showed normal Ca^{2+} transients. Our results indicate that Na^+/H^+ exchange inhibition protects against pH-dependent reperfusion injury and facilitates full recovery of cell function.

537

RESCUE OF ADULT RABBIT CARDIAC MYOCYTES FROM ISCHEMIA/REPERFUSION INJURY BY CYCLOSPORIN A AND BUTANEDIONE MONOXIME. (E. Chacon, I.S. Harper, J.M. Reece, B. Herman, and J.J. Lemasters). Dept. of Cell Biology & Anatomy and Curr. in Toxicology, Univ. of North Carolina, Chapel Hill, NC 27599, and Expl. Biol. Program, Medical Res. Council, Tygerberg 7505, South Africa.

Mechanisms underlying pH-dependent ischemia/reperfusion injury were investigated in 1-day cultured adult rabbit myocytes. Myocytes were exposed to 2.5 mM NaCN and 20 mM 2-deoxyglucose at pH 6.2 to simulate the ATP depletion, reductive stress and acidosis of ischemia. Free Ca^{2+} and mitochondrial $\Delta\Psi$ were measured in cells loaded with Fluo-3 and tetramethylrhodamine methylester using laser scanning confocal microscopy. Simulated ischemia caused cell shortening but little increase of free Ca^{2+} or decrease of mitochondrial $\Delta\Psi$ after 30 min. Reperfusion produced by inhibitor washout at pH 7.4 caused increased cytosolic and mitochondrial Ca^{2+}, hypercontraction, blebbing and mitochondrial depolarization within 1-5 min, followed by loss of viability. Increasing pH to 7.4 alone was sufficient to cause most of these changes (pH paradox). Treatment during ischemia/reperfusion with either cyclosporin A (1 μM) or butanedione monoxime (20 mM) did not prevent lethal injury. However, when cyclosporin A and butanedione monoxime were used together, Ca^{2+} loading, mitochondrial depolarization, hypercontraction and blebbing were prevented. Lethal reperfusion injury was also prevented if cyclosporin A and butanedione monoxime were added 5 min before reperfusion. These results suggest that butanedione monoxime-sensitive hypercontraction and a cyclosporin-sensitive mitochondrial permeability transition both contribute to lethal reperfusion injury.

NEURAL CONTROL OF CIRCULATION I (538-541)

538

OXYGEN-DERIVED FREE RADICALS CONTRIBUTE TO BARORECEPTOR DYSFUNCTION IN ATHEROSCLEROSIS.

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Baroreceptor (BR) sensitivity is decreased in atherosclerosis (AS). We demonstrated recently that chemically generated free radicals decrease BR sensitivity. In this study, we tested the hypothesis that endogenous oxygen-derived free radicals contribute to BR dysfunction in AS. BR activity was measured from the vascularly-isolated carotid sinus (CS) in anesthetized rabbits fed either a normal (N, n=13) or high cholesterol diet (0.5-1.0% cholesterol, n=12) for 6-8 months. AS lesions were present in the CS of AS rabbits. The CS was distended with ramp increases in pressure. BR sensitivity was decreased ($p<0.05$) in AS. The slope of the pressure-activity curve averaged 6.2±0.6 spikes/s/mmHg in AS vs. 10.8±0.8 spikes/s/mmHg in N rabbits. Maximum BR activity was also significantly less in AS vs. N rabbits (425±34 vs. 721±30 spikes/s). Exposure of the CS to the free radical scavengers superoxide dismutase (SOD, 300 units/ml) and catalase (1200 units/ml) increased maximum BR activity by 25±4% in AS rabbits (n=6, $p<0.05$) but failed to influence activity in N rabbits (-1±1%, n=5). SOD and catalase did not influence the CS pressure-diameter relation (videomicrometer, n=6) suggesting that the increase in BR activity was not caused by improved vascular distensibility. We conclude that endogenous oxygen free radicals contribute to BR dysfunction in AS. (VA, AHA, NIH)

539

ANGIOTENSIN II REVERSIBLY REDUCES CALCIUM CURRENTS IN NEONATAL RAT NODOSE NEURONS. K.Bacal and D.L.Kunze, Baylor College of Medicine, Houston, TX 77030

Angiotensin II (AII) causes cardiovascular changes when applied to the site of the first synapse of the baroreflex, and there is evidence that these effects are mediated presynaptically. As the nodose ganglia contain baroreceptor afferents, we examined the effects of AII on the calcium currents of nodose cells using a whole-cell voltage clamp protocol. We have previously shown an AII-induced activation of calcium currents in these cells; we now demonstrate a second effect on calcium current. The nodose cells were isolated from neonatal rats (1-2 days old) and grown in culture for 1-2 days before use. An intracellular solution of (in mM): 124 CsCl, 11 EGTA, 1 $CaCl_2$, 2 $MgCl_2$, 10 HEPES was used, with 50 µg/ml nystatin to provide a "perforated patch". The bath solution contained 139 tetraethylammonium chloride, 2 $CaCl_2$, 2 glucose, 10 HEPES, 5 4-aminopyridine. Under these conditions, perfusion with 10 nM AII reduced maximum calcium current by 43 ± 17% (n=25). The AII effect was completely reversible upon re-perfusion with bath and could be abolished by 100 nM losartan, a specific antagonist for the AT_1-type AII receptor. Incubation of the cells in pertussis toxin (PTX) likewise eliminated the effect, indicating that a G-protein is involved in transduction of the signal. Application of 1 µM ω-conotoxin GVIA (CTX) markedly diminished calcium currents in these cells, and the remaining current was unaffected by AII. For these reasons, we propose that the AII-induced inhibition of calcium current in neonatal rat nodose neurons is mediated by the AT_1 receptor, coupled to CTX-sensitive ion channels through a PTX-sensitive G-protein. Supported by NIH HL-36840.

540

CALCIUM INFLUX MEDIATES MECHANO-SENSITIVE INTRACELLULAR CALCIUM TRANSIENTS IN NODOSE SENSORY NEURONS. R. V. Sharma, R.E Wachtel, G. Hajduczok, M. W. Chapleau, L. Fankhauser, R. C. Bhalla and F. M. Abboud. The Univ. of Iowa Coll. of Med. and Dept. Vet. Aff. Med. Ctr., Iowa City, IA 52242.

We have recently shown that mechanical stimulation increases intracellular calcium concentration ($[Ca^{2+}]_i$) in sensory neurons isolated from the nodose ganglion, which contains baroreceptor perikarya. In the present study we have tested the hypothesis that influx of calcium mediates the response to mechanical stimuli. Enzymatically isolated rat nodose neurons in primary cultures were mechanically stimulated by probing with a blunt pipette and $[Ca^{2+}]_i$ was quantitated using a fluorescence microscopic digital image analysis system and Fura-2. In physiological buffer solution (PBS) containing 1.8 mM calcium, mechanical stimulation increased $[Ca^{2+}]_i$ in 31 of 42 neurons, from 129±6 to 708± 90 nM. In a number of experiments mechanical stimulation of one neuron resulted in intercellular propagation of the transient rise in $[Ca^{2+}]_i$ to a second neuron in contact with the stretched neuron (125±6 vs. 810±128 nM, n=15). Mechanical stimulation failed to increase $[Ca^{2+}]_i$ in nodose neurons in calcium free PBS (104±7 vs. 154±29 nM, n=12) or in the presence of gadolinium (10^{-3} M, n=4), an inhibitor of stretch activated (SA) ion channels. We conclude that in nodose neurons the increase in $[Ca^{2+}]_i$ transient is due to an influx of calcium, possibly triggered by opening of SA channels, and that this increase in $[Ca^{2+}]_i$ is propagated to some adjacent neurons in contact with the stretched neurons. Supported by USPHS Grants HL 14388 and HL 44546.

541

A MEMBRANE MODEL OF THE AORTIC BARORECEPTOR NEURON

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We have identified and characterized the essential ion channel currents in aortic baroreceptor neurons (ABN) of neo-natal and juvenile rat. The isolation of these neurons from the milieu of cellular structures within excised nodose ganglia was accomplished using a combination of enzymatic dispersion and fluorescence identification techniques in selected juvenile cells. Using a cellular patch clamp technique under voltage clamp conditions, we have identified two Na^+ currents, two Ca^{2+} currents and four K^+ currents. In addition, recordings were made of somatic action potentials using a variety of current stimulus waveforms. This data was used in the development of a Hodgkin-Huxley type model of the cell. Identification of model parameters associated with an individual ion current equation was accomplished using a nonlinear least-squares parameter estimation algorithm. This system of equations was assembled into a comprehensive ion current membrane model of the rat ABN. A lumped fluid model consisting of three separate well-stirred compartments containing different concentrations of Na^+, Ca^{2+}, and K^+ was coupled to this membrane model. The three compartments are: (a) an intracellular fluid space describing lumped ion concentrations and protein binding sites for Ca^{2+} on a calmodulin type buffer, (b) an annular fluid space describing local ion accumulation within the perineuronal volume and (c) a large extracellular volume where all ionic concentrations are assumed to be constant. The resultant cell model is capable of accurately reproducing the electrophysiological response of ABNs under a variety of voltage- and current-clamp protocols.